Monoclonal Antibody Labeling for Cytokeratins and Filaggrin in the Human Pilosebaceous Unit of Normal, Seborrhoeic and Acne Skin

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The distribution of cytokeratins and filaggrin in human pilosebaceous unit was investigated in specimens obtained from normal (n = 15), seborrhoeic (n = 6), and acne skin (n = 6), using the monoclonal antibodies CK8.12, CK8.13, CK4.62, CK8.60, KL_1, PKK_2, RPN 1160, and an antibody for filaggrin. The type and amount of cytokeratin content was correlated with the stage of cell differentiation in these three skin types. In all specimens studied the sebocytes. The sebaceous duct cells, and the infundibular cells contained cytokeratins, no clear differences were found between normal, seborrhoeic, and acne skin. During sebocytic maturation the amount and type of cytokeratin content changed gradually and the labeling pattern was partly different compared to the interfollicular epidermal pattern. In the sebaceous duct and the infundibulum, the labeling pattern using KL_1, CK8.12, and CK8.13 was similar to that seen in interfollicular epidermis, whereas labeling with CK8.60 and PKK_2 was different. These findings indicate that sebaceous duct and infundibular cells express transitional patterns of differentiation between epidermal keratinocytes and sebocytes. Filaggrin was expressed only in some sebaceous duct cells and in infundibular cells. In seborrhoeic and in acne skin, however, the reactivity of antibody to filaggrin was more intense and was already observed in the lower parts of the sebaceous duct and the infundibulum. Although no filaggrin was found in the intermediate cells of the sebaceous duct and the infundibulum in normal skin, these cell types clearly contained filaggrin in seborrhoeic and acne skin. *J Invest Dermatol 91:566–571, 1988*

The sebaceous gland is composed of several lobuli, emerging in a common duct through which the gland communicates with the follicular opening (Fig 1). Each lobulus is composed of sebocytes showing different stages of sebum production. Basically, five types of sebocytes were identified, representing progressive steps of cell maturation and of lipid synthesis and accumulation: a) undifferentiated (basal) cells, b) early differentiated cells, c) advanced differentiated cells, d) fully differentiated cells, and e) mature sebocytes [1]. The cell size and lipid content increase dramatically during the development of undifferentiated basal cells into mature sebocytes (Fig 2). Electron microscopic studies have shown that undifferentiated basal cells of the sebaceous epithelium contain abundant cytoplasmic filaments, organized either in a fine meshwork or forming thin bundles [2]; they are particularly prominent in the desmosomal region; and their amount decreases during the sebocytic maturation process [2].

The sebaceous duct, a transitional zone between follicular channel and the lipid producing cells, is formed by a stratified epithelium in continuity with the outer hair root sheath and the interfollicular epidermis (Fig 1). Similar to sebocytes, abundant bundles of filaments and few amounts of lipid droplets were found in the cytoplasm of the sebaceous duct cells [2,3].

Immediately above the sebaceous duct orifice, the infundibular epithelium undergoes partial keratinization with production of small keratohyaline granules and formation of a thin, fragile, and imperfect horny layer [3]. In wound healing, undifferentiated sebaceous cells move from the infudibulum to the interfollicular epidermis for epidermal repair; correspondingly, interfollicular keratinocytes maintain the capacity to differentiate into sebaceous cells [4]. Biochemical studies have shown the presence of cytokeratins in interfollicular epidermis and in adnexal glands, including the pilosebaceous unit [5].

Cytokeratins are important markers and may be helpful for evaluating the clonal origin and the stage of keratinocytic differentiation [6–8]. Besides cytokeratins, the presence of filaggrin ("filament aggregating protein") is a distinct marker of the terminal phase of the epidermal keratinization process [9]. Filaggrin most probably acts as an interfilamentous matrix protein, contributing to the assembling of keratin filaments into macrofibrils [10]. Its amount and distribution are altered in disorders of keratinization such as ichthyosis vulgaris [11] and epidermolytic hyperkeratosis [12].

In the pathogenesis of acne, the increased sebum excretion rate [13,14], hormonal influences [15,16], alterations of size and function of the follicular channel [17], and secondary bacterial colonization [17] are regarded as essential factors; in addition, some authors believe that abnormal keratinization of the follicular channel could play the major role in the development of acne lesions [17,18].

In this paper the distribution of cytokeratins and of filaggrin in the sebaceous gland and the infundibular area of normal, seborrhoeic, and acne skin was studied and; in addition, their alterations during sebaceous cell maturation were described.

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Abbreviations:
APAAP: alkaline phosphatase and monoclonal anti-alkaline phosphatase
IgG: immunoglobulin class G
McAbs: monoclonal antibodies
PBS: phosphate buffered saline

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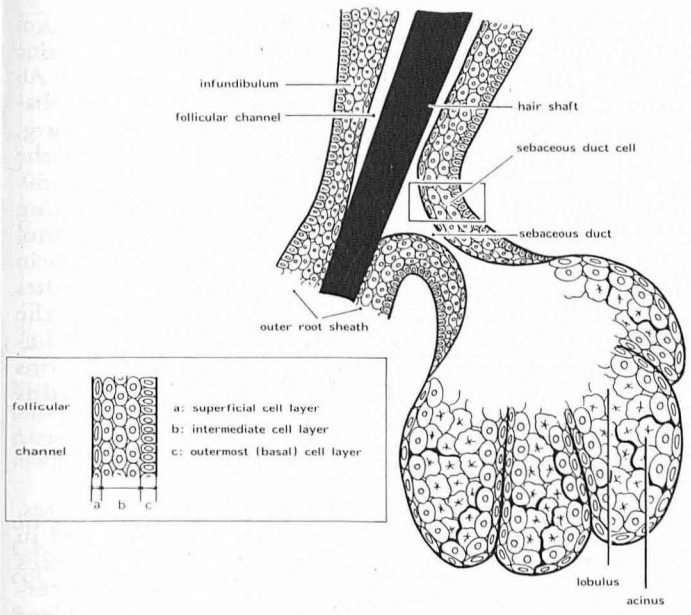

Figure 1. A diagram of the normal pilosebaceous unit.

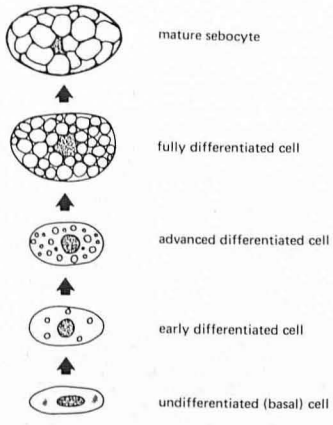

Figure 2. Types of sebocytes during maturation.

Table I. Specificity of McAbs Used and Their Labeling Pattern of the Normal Epidermis

McAb	Specificity for Cytokeratin(s)	Labeling pattern of the normal epidermis	Reference
KL_1	1, 2, 5, 6, 7, 8, 10, 11, 17	Suprabasal layers	[19]
CK8.60	10/11	Suprabasal layers	[20]
CK8.12	13, 16	Basal layers	[20]
CK8.13	1, 5, 6, 7, 8, 10, 11, 18	All layers	[21]
CK4.62	19	Negative	[22]
PKK_2	7, 16, 17, 19	Basal layer	[23]
RPN1160	18	Negative	[24]

MATERIALS AND METHODS

Skin Specimens Skin specimens were obtained by surgical excision from the upper back of 27 young individuals, immediately frozen in liquid nitrogen, and stored at −20°C until used. Of 27 individuals, 15 had normal skin, six seborrhoeic skin, and six acne skin.

Table II. Reactivity of Cytokeratin and Filaggrin McAbs in Sebaceous Gland, Sebaceous Duct, and the Infundibular Area

Type of Cells (anatomical site)	Differentiation	Monoclonal Antibodies (McAbs)							Filaggrin			APAAP (neg. control)
		PKK_2	CK8.13	KL_1	CK8.60	CK8.12	RPN1160	CK4.62	normal	seborrhea	acne	
Sebaceous cells	Mature cells	++	+	−	−	−	−	−	−	−	−	−
	Fully differentiated cells	++	+	−	−	−	−	−	−	−	−	−
	Advanced differentiated cells	++	+	+	−	−	−	−	−	−	−	−
	Early differentiated cells	+	+	+/++	−/+	−	−	−	−	−	−	−
	Undifferentiated (basal) cells	+	+/++	++	−/+	−	−	−	−	−	−	−
Sebaceous duct cells	Superficial cells	++/+++	++	+++/+++	+	−	−	−	+	++	++	−
	Intermediate cells	++	++	+++/++++	+++	−	−	−	−	−/+	−/+	−
	Outermost (basal) cells	+	++	−	−	++/+++	−	−	−	−	−	−
Infundibular Epithelial Cells	Granular layer cells	++/+++	+++	+++/++++	++	−	−	−	++	++/+++	+++	−
	Intermediate cells	++/+++	++	+++/++++	+++	−	−	−	−	−/+	−/++	−
	Outermost (basal) cells	+	++	−	−	++/+++	−	−	−	−	−	−

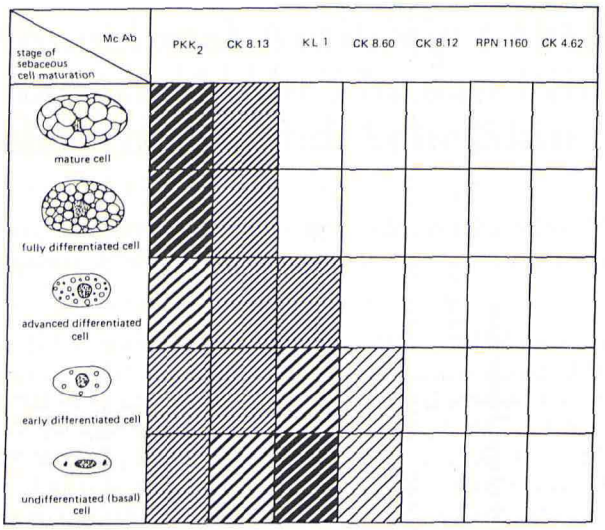

Figure 3. Reactivity of cytokeratin McAbs in sebaceous cells in various *stages of differentiation. The reactive intensity of McAbs is classified into* four degrees as follows. Very strong: ▨ ; strong reaction: ▨ ; moderately strong reaction: ▨ ; and weak reaction: ▨

Monoclonal Antibodies (McAbs) To study the cytokeratins and filaggrin distribution, the following McAbs were selected: KL_1 (Immunotech, Marseilles, France); CK8.60, CK8.12, CK8.13, and CK4.62 (Bio Yeda, Rehovot, Israel); PKK_2 (Labsystems, Helsinki, Finland); the monoclonal anti-simple epithelia RPN 1160 (Amersham, Buckinghamshire, England); and the McAb against human filaggrin Biotech, Stoughton, USA. The specificity of McAbs used and their labeling pattern of the normal epidermis are shown in Table I [19–24].

Methods Immunocytochemical techniques using alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP) were performed according to standard techniques [25,26] in 5–7-μm frozen sections adhered to glass slides. Briefly, after a 5-min acetone fixation, the sections were incubated with the appropriate McAb diluted in 0.1 M phosphate buffer saline (PBS) for 30 min. Incubations were made with rabbit antimouse-IgG (Dianova, Hamburg, Federal Republic of Germany) diluted to 1:100 and with the APAAP complex (Dako, Glostrup, Denmark) diluted to 1:50. These two steps were performed twice to intensify the labeling reaction that was visualized with a solution containing naphthol AS-BI sodium phosphate (Sigma, St. Louis) and new fuchsin (Merck, Darmstadt, Federal Republic of Germany). Careful washes with PBS were performed between each incubation step and the sections were never allowed to dry. After counterstaining with hemalum, the slides were mounted with glycerol gelatin. Sections processed after omission of the incubation with the McAb served as negative controls.

RESULTS

The distribution of cytokeratins and filaggrin in human sebocytes, sebaceous duct cells, and infundibular epithelia is summarized in Table II, according to their labeling with McAbs. No differences were found between normal, seborrhoeic, and acne skin in cytokeratin content, whereas significant differences were seen in the filaggrin McAb binding.

Sebocytes (Sebaceous Cells) Distinct patterns and intensities of McAbs labeling were obtained, depending on the stage of sebocytic differentiation and the particular McAb used (Fig 3). All undifferentiated, early differentiated, and advanced differentiated sebocytes were consistently labeled by the McAbs PKK_2, CK8.13, and KL_1, showing diffuse cytoplasmic staining. Undifferentiated and early differentiated sebocytes showed, in addition, some labeling with McAb CK8.60; the pattern of this labeling, however, was diffuse and weak; fully differentiated and mature sebocytes were only labeled by McAbs PKK_2 and CK8.13. Maximal labeling intensity for McAbs CK8.13 and KL_1 was seen in undifferentiated (basal) cells and was found to decrease during cell maturation. On the other hand, the staining intensity of PKK_2 progressively increased from

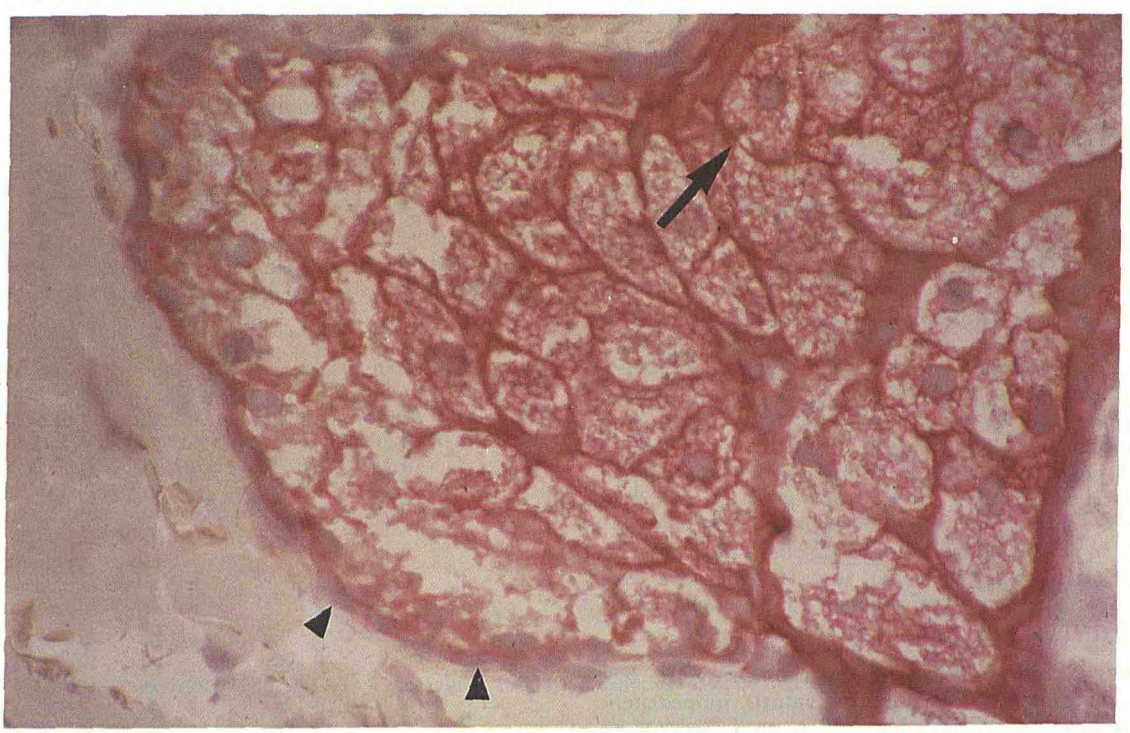

Figure 4. Immuno-alkaline phosphatase staining with PKK_2 in the sebaceous gland of seborrhoeic skin: the undifferentiated *(basal)* cells *(arrowhead)* are weakly positive, but positivity is more marked in the fully differentiated cells *(arrow)* (×112).

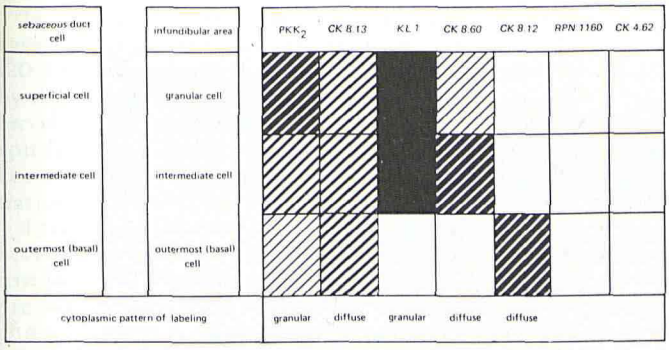

Figure 5. Reactivity of cytokeratin McAbs in the sebaceous duct and the infundibular area. The reactive intenstity of McAbs is classified into four degrees as follows. Very strong reaction: ▬ ; strong reaction: ▨ ; moderately strong reaction: ▨ ; and weak reaction: ▨

undifferentiated to mature cells (Fig 4). The McAbs CK8.12, CK4.62, RPN 1160, and filaggrin failed to bind to sebaceous gland cells.

Sebaceous Duct Cells Three cell layers were considered in the sebaceous duct: the superficial cell layer, an innermost layer facing the follicular channel; the intermediate cell layers, interposed between the superficial cell layer and the outermost (basal) layer; and the outermost (basal) layer (Fig 1). Distinct patterns and intensities of McAbs labelings were observed in each of these layers (Fig 5).

The McAb PKK_2 labeled all three layers with increasing intensity

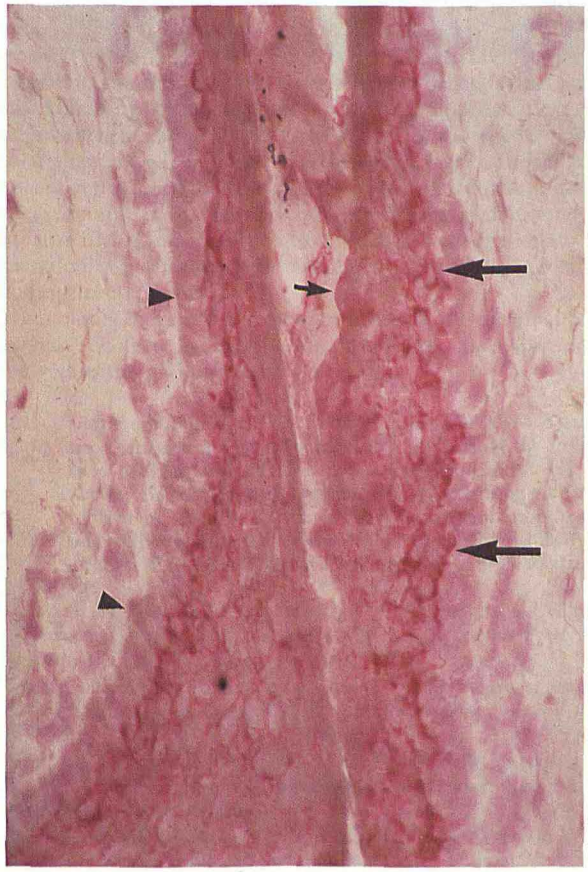

Figure 6. Immuno-alkaline phosphatase staining with CK8.60 in the sebaceous duct and the infundibulum of acne skin: the superficial cells *(small arrow)* are weakly labeled and the intermediate cells *(large arrow)* are strongly labeled, while the outermost *(basal)* cells *(arrowhead)* are unlabeled.

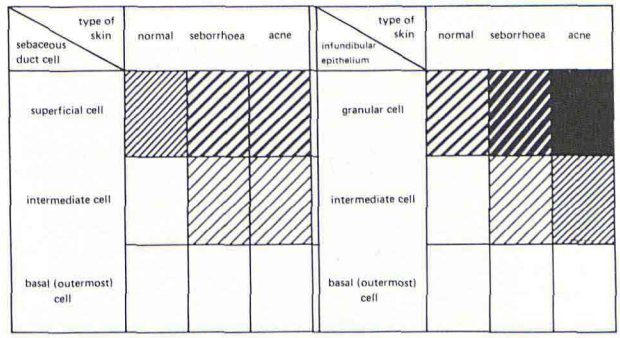

Figure 7. Reactivity of McAb filaggrin distribution in sebaceous duct cells and infundibular epithelium on normal, seborrhoeic, and acneic skin. The reactive intensity of McAb filiggrin is classified into five degrees as follows. Very strong reaction: ▬ ; strong reaction: ▨ ; moderately strong reaction: ▨ ; weak reaction: ▨ ; very weak reaction: ▨

from the basal to the superficial layers, while the McAb CK8.13 labeled them homogeneously. Most intense labeling was seen with McAb KL_1 in the intermediate and superficial cell layers. These two layers, in addition, were intensely marked by McAb PKK_2 and CK8.60, while McAb CK8.12 only stained the basal cell layer. The McAb CK8.60 showed the highest binding intensity in the intermediate layer (Fig 6). The cytoplasmic patterns of labeling were granular to McAbs PKK_2 and KL_1 and diffuse to McAbs CK8.13, CK8.60, and CK8.12. The McAbs CK4.62 and RPN 1160 failed to bind.

The binding of filaggrin McAb to sebaceous duct cells was different in specimens obtained from normal, seborrhoeic, and acne skin (Fig 7). In normal skin only the superficial cell layer was labeled, presenting diffuse cytoplasmic staining with fine, intensely labeled granules. In acne and seborrhoeic skin the superficial cells and the innermost cells of the intermediate layers were strongly labeled. The diffuse labeling was more intense in the cells of the superficial layers, showing, in addition, large amounts of strongly reactive cytoplasmic macroaggregates.

Infundibular Epithelial Cells The anticytokeratin McAb labeling patterns observed in the infundibular area were similar to those described in sebaceous duct cells (Fig 5). However, the binding of filaggrin was strikingly different between normal, seborrhoeic, and acne skin. In normal skin, only the granular cells showed diffuse cytoplasmic labeling, and small granular deposits were also observed (Fig 8*a*). In seborrhoeic (Fig 8*b*) and acne skin (Fig 8*c*) the granular cells showed strong labeling, and the innermost cells of the intermediate layer were also clearly marked. In general, the labeling with anticytokeratin and filaggrin McAbs was more intense in the infundibular epithelium than in the sebaceous duct cells. The intensity of binding increased from the intermediate to the most superficial cell layers and its pattern changed from diffuse or fine granular in the intermediate layers to continuous supranuclear capping in the upper superficial cells.

DISCUSSION

These results clearly demonstrate that cytokeratins are present in varying amounts in all sebocytes, sebaceous duct cells, and infundibular keratinocytes, whereas filaggrin is only expressed by some cells of the sebaceous duct and the infundibular area. No differences were found between normal, seborrhoeic, and acne skin using anticytokeratin McAbs; evidently, the early phases of keratinization leading to formation of cytokeratins are not clearly disturbed in acne.

Distinct staining patterns were observed in each particular anatomical site of the pilosebaceous unit depending on the McAb used, but McAb CK4.62 and RPN 1160, which recognize cytokeratins 19 and 18 [22,24], failed to bind to the glandular or epithelial cells. Biochemical techniques also revealed that these cytokeratins do not occur in human sebaceous glands and in hair follicles [27], in accordance with our findings.

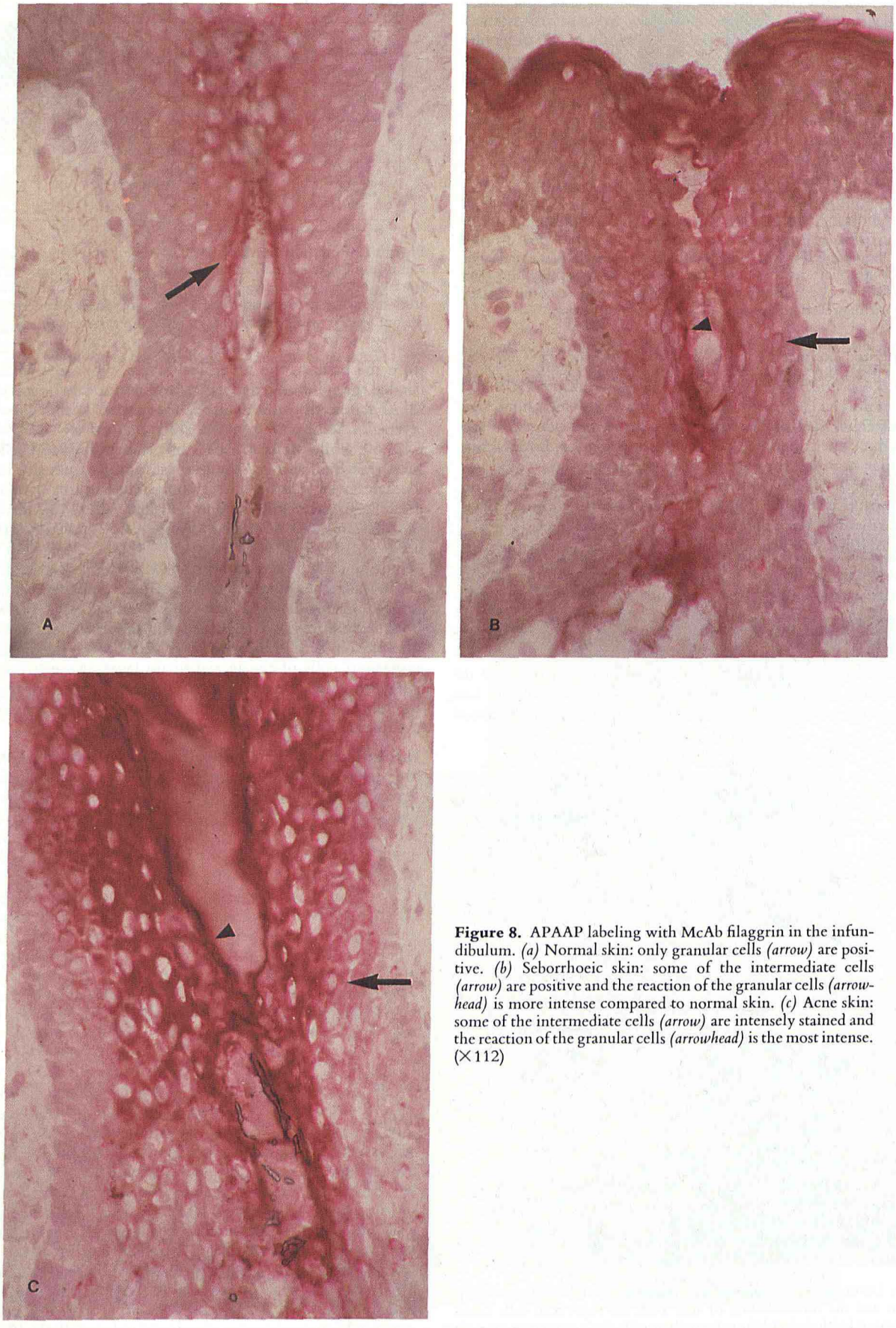

Figure 8. APAAP labeling with McAb filaggrin in the infundibulum. *(a)* Normal skin: only granular cells *(arrow)* are positive. *(b)* Seborrhoeic skin: some of the intermediate cells *(arrow)* are positive and the reaction of the granular cells *(arrowhead)* is more intense compared to normal skin. *(c)* Acne skin: some of the intermediate cells *(arrow)* are intensely stained and the reaction of the granular cells *(arrowhead)* is the most intense. (×112)

It has been reported that during epidermal differentiation the basal cell cytokeratins, predominantly labeled by CK8.12 and PKK_2 [20,23], disappear and that the suprabasal cell cytokeratins labeled by KL_1 and CK8.60 [19,20] are induced. In addition, CK8.13 [21], a broadly cross-reacting McAb, labels all epidermal layers homogeneously. These findings indicate that the types and amounts of cytokeratin content largely depend on the stage of epidermal differentiation [4–6].

In the sebaceous gland, we also found that labeling with anticytokeratin McAbs may vary gradually during sebocytic maturation, similar to the interfollicular epidermis. However, cytokeratin expression during the maturation of sebocytes does not display drastic changes similar to those found in basal and suprabasal layers of interfollicular epidermis, indicating that sebocytic differentiation is a gradually progressing process. Moreover, different labeling patterns were obtained during sebocytic differentiation compared to those seen during keratinocytic differentiation in interfollicular epidermis.

In the sebaceous duct and the infundibulum, distinct changes were found between basal and suprabasal layers, and different cytokeratin patterns were found in these areas, compared to those seen in the sebaceous gland and the interfollicular epidermis. Particularly PKK_2, a marker of basal epidermal keratinocytes, which also stained all sebaceous duct cells and infundibular epithelia. These findings indicate that the sebaceous duct and the infundibulum represent transitional areas between epidermal keratinocytes and sebocytes, suggesting a bimodality of these cells to differentiate into both directions.

In contrast to the rather unaltered pattern of cytokeratin content in normal, seborrhoeic, and acne skin, the expression of filaggrin in human sebaceous apparatus was clearly different, depending on the type of skin studied. In all specimens from normal, seborrhoeic, and acne skin filaggrin was present in the superficial cells of the sebaceous duct and in the granular cells of the infundibulum. In addition, seborrhoeic and acne skin also revealed considerable amounts of filaggrin in the intermediate layers of the sebaceous duct and in the infundibulum, indicating a premature terminal keratinization process in these areas. This may result in the obstruction of the follicular channel, which is one of the essential factors for the pathogenesis of acne. Correspondingly, in electron microscopic studies an increased number of keratohyaline granules was seen in acne skin [3]. Because filaggrin is regarded to be related with the keratohyaline granules [28], this observation supports the immunocytochemical findings presented here.

We conclude, therefore, that abnormalities of the terminal phase of keratinocytic differentiation in the infundibular area may indeed be involved in the pathogenesis of acne.

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